



Research activities for targeted therapies in cancer

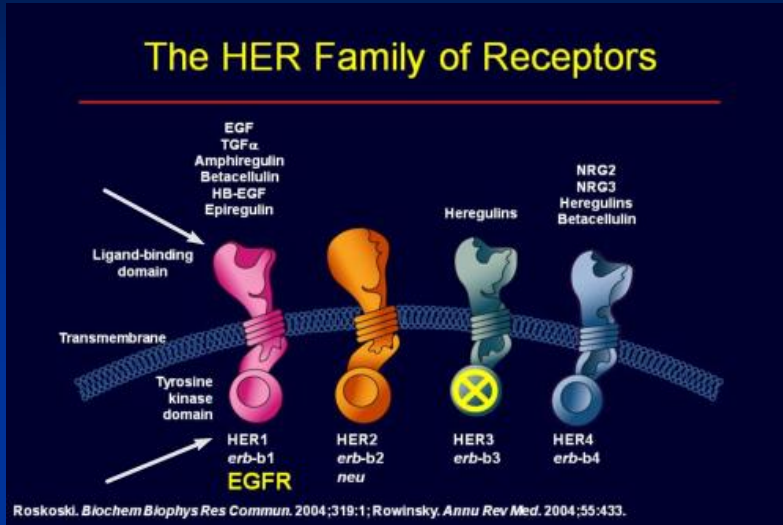
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Aims of our research

- Biotargeting Agents: **Multi-targeting** of receptors and/or intracellular kinases for **enhancing** their anti-tumour effect
- Investigation of **pathways** affected by biotargeting agents
- Identification of **new molecular targets**
- Identification of sensitive and specific **prognostic and predictive biomarkers**

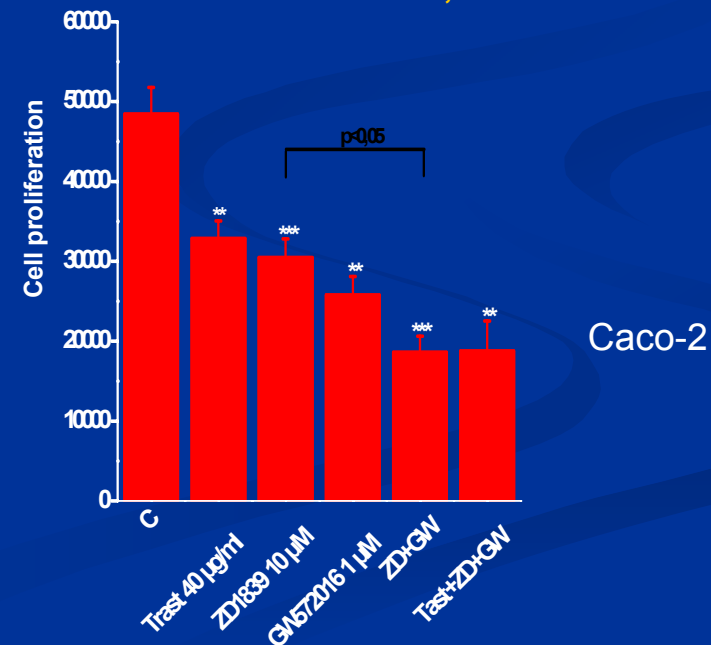
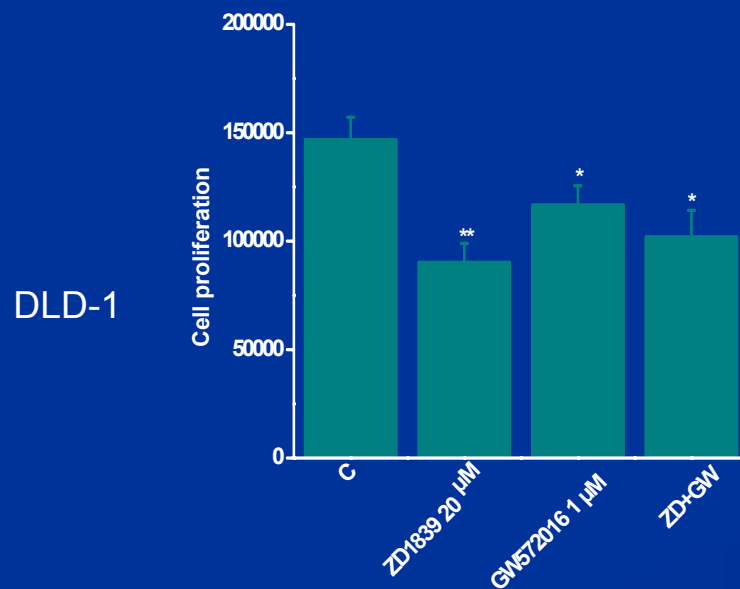
A. *In vitro* targeting of receptors and intracellular kinases in cancer

I. Combined targeting of HER family in colon cancer

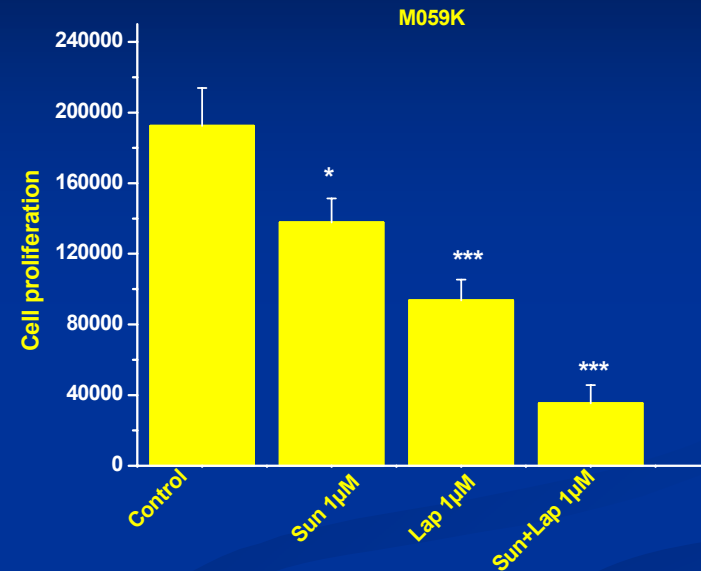
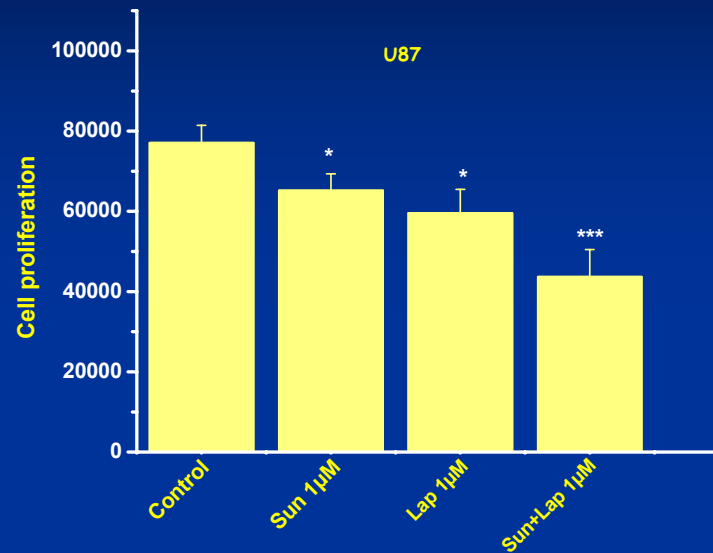


- Synergistic effect of Gefitinib (Iressa) and Lapatinib (Tyverb) in Caco-2 cells.
- However, combined targeting of intracellular and extracellular domain of HER-2 does not improve the anti-tumour efficacy of tested agents.

Giannopoulou E, Antonacopoulou A, Floratou K, Papavassiliou AG, Kalofonos HP. Dual targeting of EGFR and HER-2 in colon cancer cell lines. **Cancer Chemother Pharmacol.** 2009; 63:973-81.



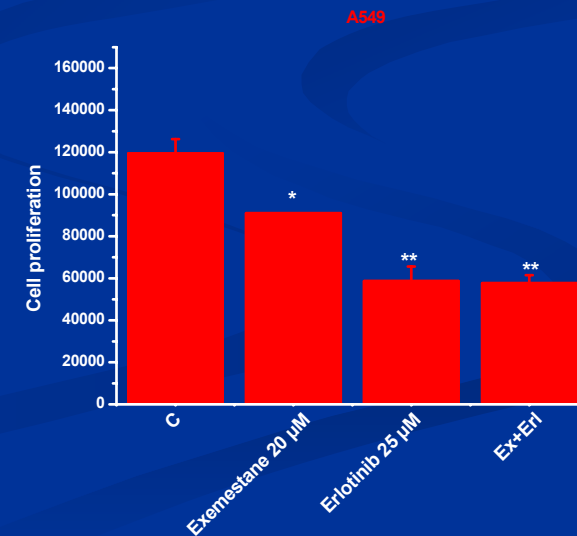
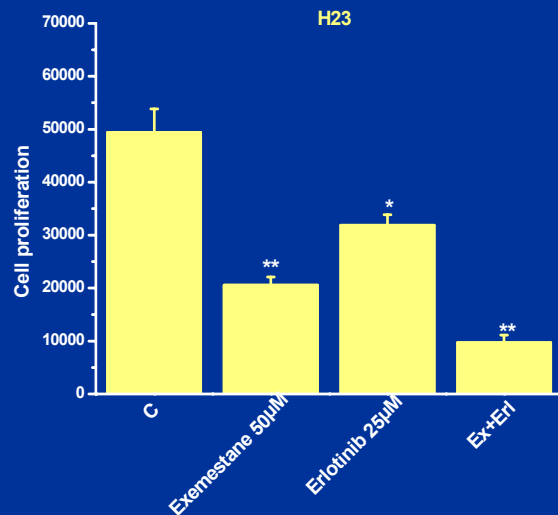
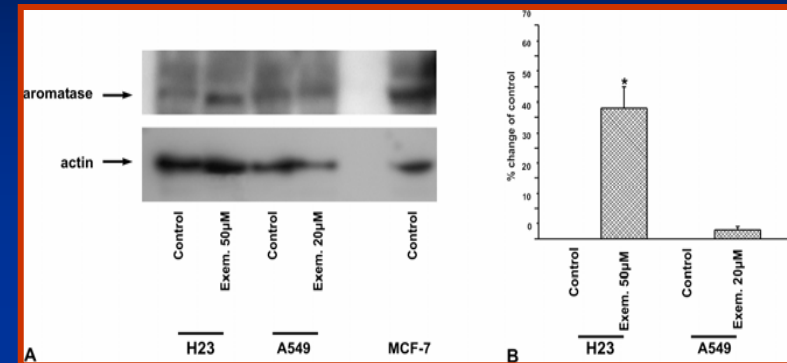
II. Combined targeting of HER1&2, VEGFR and PDGFR in glioblastoma cells



- Sunitinib plus lapatinib have a **synergistic effect** in cell proliferation of glioblastoma cells.
- An inhibitory effect was observed in cell migration, too.
 - Giannopoulou E, Dimitropoulos K, Argyriou AA, Koutras AK, Dimitrakopoulos F, Kalofonos HP. An in vitro study, evaluating the effect of sunitinib and/or lapatinib on two glioma cell lines. **Invest New Drugs, 2010; 28:554-60.**
 - Dimitropoulos K, Giannopoulou E, Argyriou AA, Kalofonos HP. The effects of anti-VEGFR and anti-EGFR agents on glioma cell migration through implication of growth factor with integrins (submitted).

III. Combined targeting of HER1 and Aromatase in non small cell lung cancer (NSCLC) cells

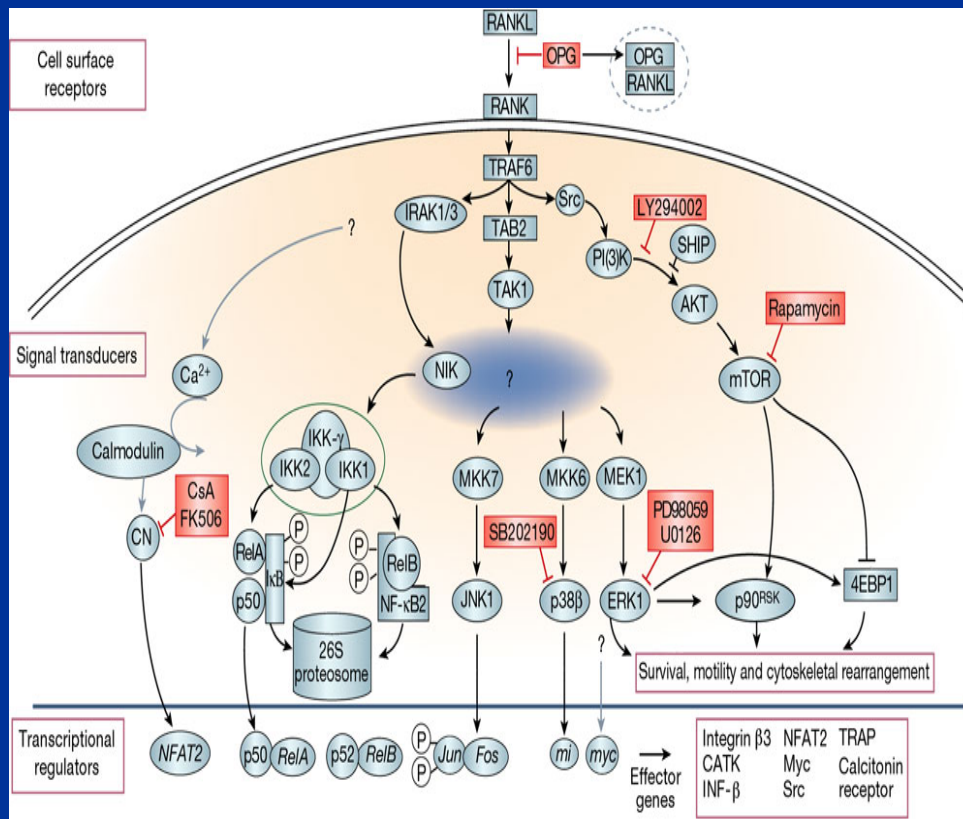
- The combination of Exemestane and Erlotinib exerts a **synergistic** anti-tumour effect in H23 cells.
- Koutras A, Giannopoulou E, Kritikou I, Antonacopoulou A, Evans TR, Papavassiliou AG, Kalofonos H. Antiproliferative effect of exemestane in lung cancer cells. **Mol Cancer, 2009;8:109 -121**
- Kritikou I, Giannopoulou E, Kalofonos H. Combined targeting of Aromatase and Epidermal Growth Factor Receptor in Non-Small-Cell Lung Cancer (submitted).



IV. Regulation of breast cancer cells by the Receptor Activator of Nuclear Factor NF κ -B (RANK)

- RANK is a member of the tumor necrosis factor (TNF) receptor superfamily
- Binding of RANK-Ligand to RANK, stimulates signaling pathways via the activation of NF κ -B, JNK and p38 kinase

- The RANKL/RANK system is implicated in:



- Bone remodeling. Activation of osteoclasts

- Immune system. Activation and survival of Dendritic Cells

- Central Nervous System. Female thermoregulation and the central fever response in inflammation

- At gestation. RANK $^{-/-}$ mice are unable to develop lactating mammary glands during pregnancy

Nature 423, 337-342 (2003)

V. The role of **Drosha**, **Dicer** and **Ago2**, central components of the miRNA-machinery in selected malignancies

Leiomyosarcomas

- Studies on SK-LMS leiomyosarcoma cell lines and on human tissue microarrays (n=101 cases)

Methods: IHC on human samples; RT-PCR, Immunofluorescence and Western blotting on cell lines

Results:

- These endonucleases are expressed in leiomyosarcomas being up-regulated in high-grade tumors
- Dicer expression levels are **strongly** associated with grade and metastatic potential

Colon cancer

- Studies on human samples (n=102) and 3 different colon cancer cell lines (Caco-2, DLD-1, HT-29)

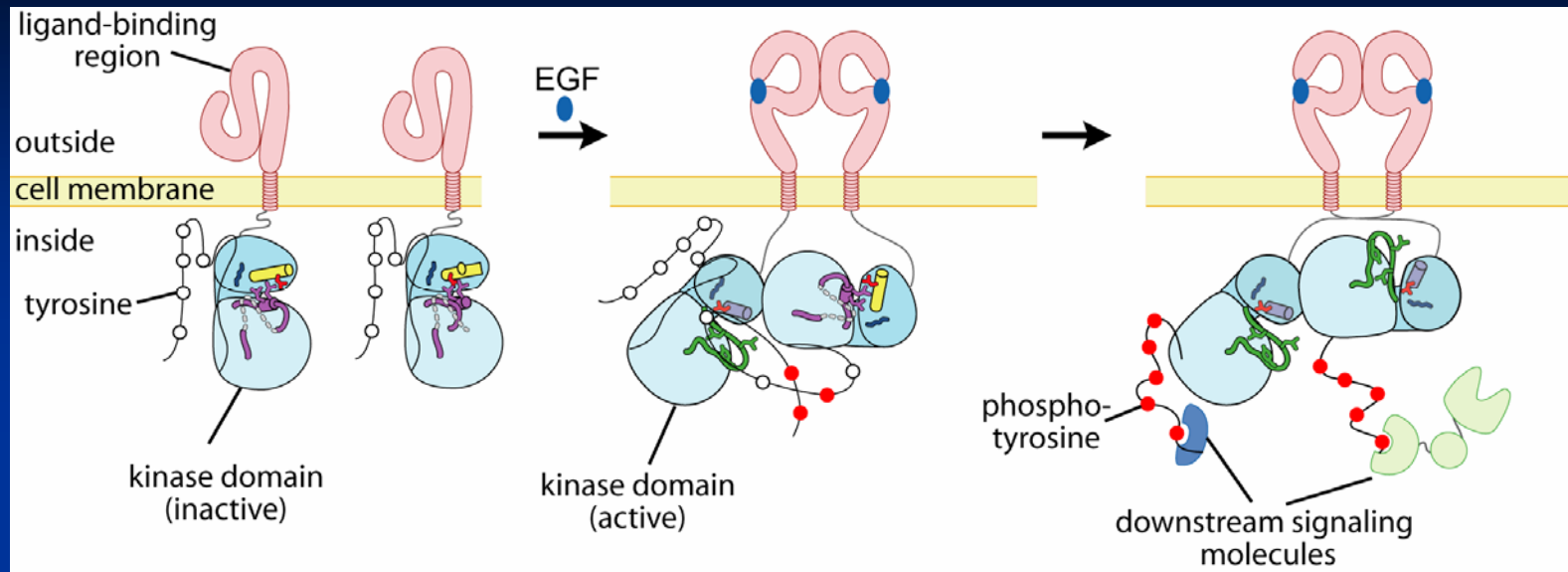
Methods: IHC and RT-PCR on human samples; RT=PCR, IF, Western-Blotting on the cell lines

Results:

- These endonucleases are expressed in human colon cancer
- Protein and mRNA expression levels are associated with advanced tumor stage (Duke's classification)

B. Biomarkers with prognostic value

HER3



EUROPEAN JOURNAL OF CANCER 43 (2007) 2602-2611



available at www.sciencedirect.com

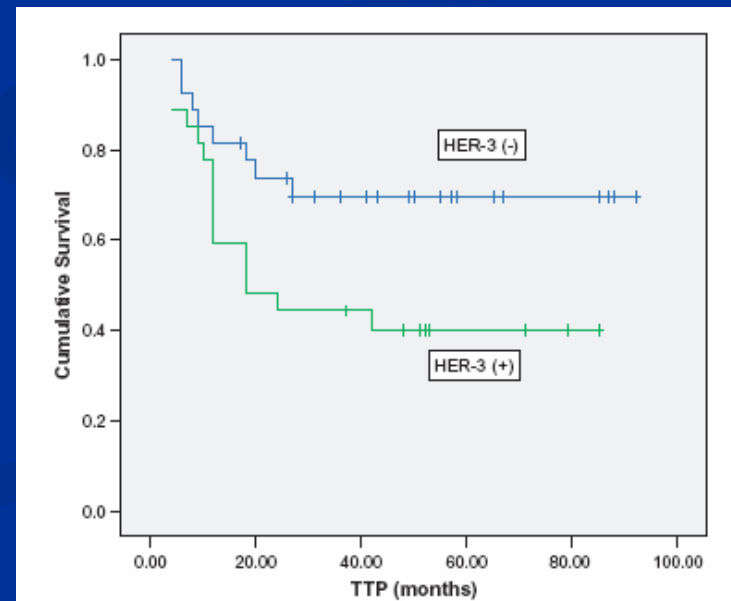
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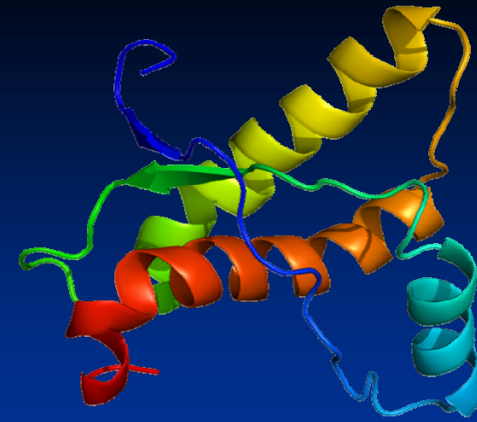


HER-3 in colorectal tumourigenesis: From mRNA levels through protein status to clinicopathologic relationships

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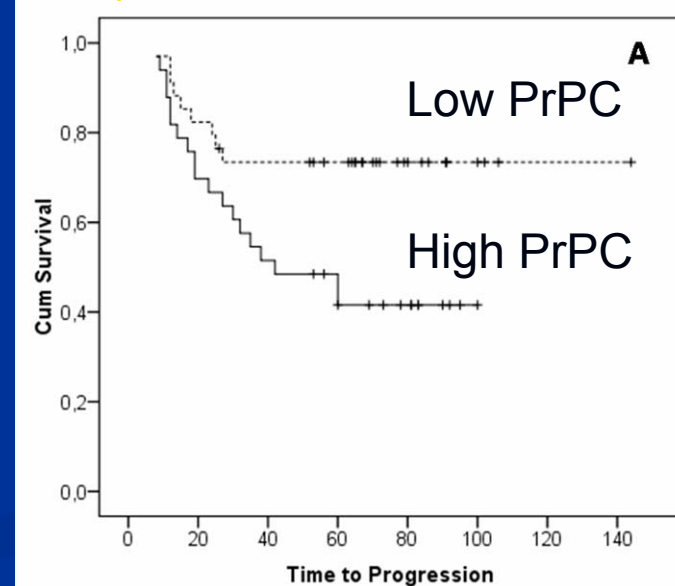
PRION PROTEIN, PrPC



- Role in cell adhesion, signal transduction, regulation of copper metabolism, protection from apoptosis and oxidative stress.
- Pathogenic misfolded form (PrP^{Sc}), involved in neurodegenerative spongiform encephalopathies.
- Higher expression in neoplastic tissue compared to normal.

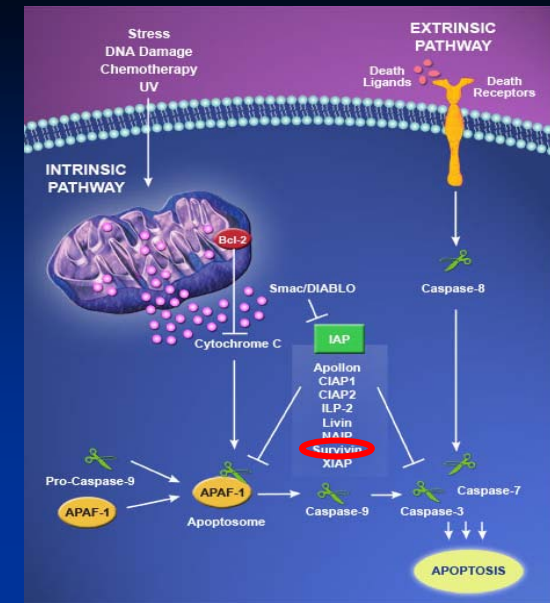
Antonacopoulou A, Palli M, Marousi S, Dimitrakopoulos FI, Kyriakopoulou U, Tsamandas AC, Scopa CD, Papavassiliou AG, Kalofonos HP. Molecular Carcinogenesis, 2010;49:693-699

High protein levels are related to **shorter time to relapse**.



SURVIVIN (BIRC5)

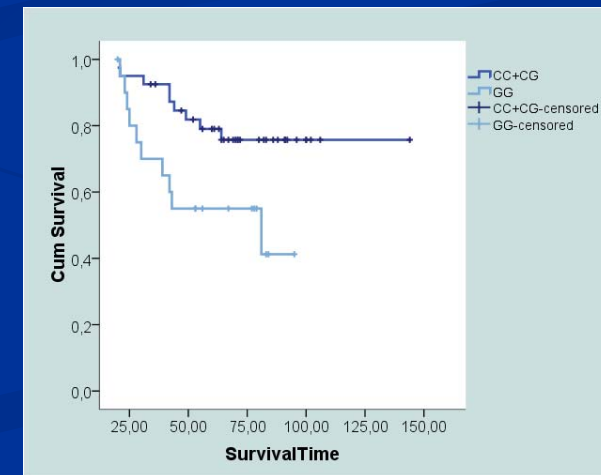
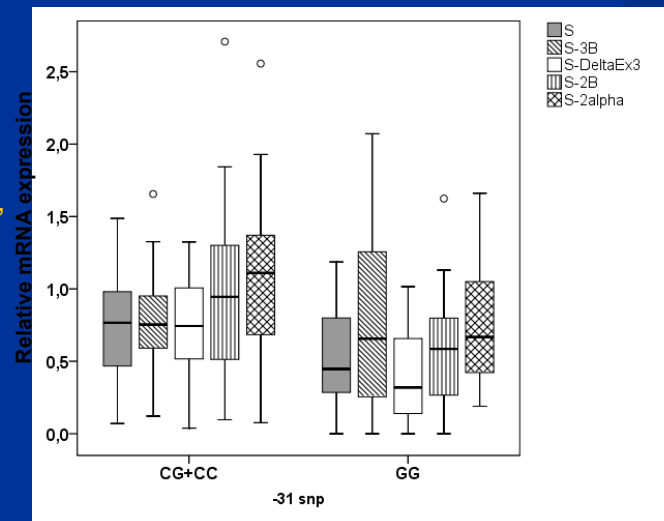
- Role in cell division and apoptosis
- Expressed during embryonic development but not in most differentiated tissues
- Increased expression in cancer
- Resistance factor to therapy
- Five isoforms with different functions
- Promoter polymorphism: -31 G/C



-31G/C snp correlates with survival rate

-31G/C snp influences expression only in tumor tissue

Antonacopoulou AG, Floratou K, Bravou V, Kottorou A, Dimitrakopoulos FI, Marousi S, Stavropoulos M, Koutras AK, Scopa CD, Kalofonos HP. The survivin -31 snp in human colorectal cancer correlates with survivin splice variant expression and improved overall survival. *Anal Cell Pathol (Amst)*. 2010



(95%CI: 94.5-121.3, $p=0.019$)

Highly neurotoxic agents

Cisplatin
Oxaliplatin
Thalidomide
Bortezomib
Suramin
Gemcitabine
Etoposide
Carboplatin
Ifosfamide
Interferon- α
Misonidazole
Procarbazine

Paclitaxel
Vincristine
Epothilones
Docetaxel
Vinblastine
Vinorelbine
Vindesine
Hexamethylmelamine
Ara-C, Ara-A, Ara-G

Low neurotoxicity

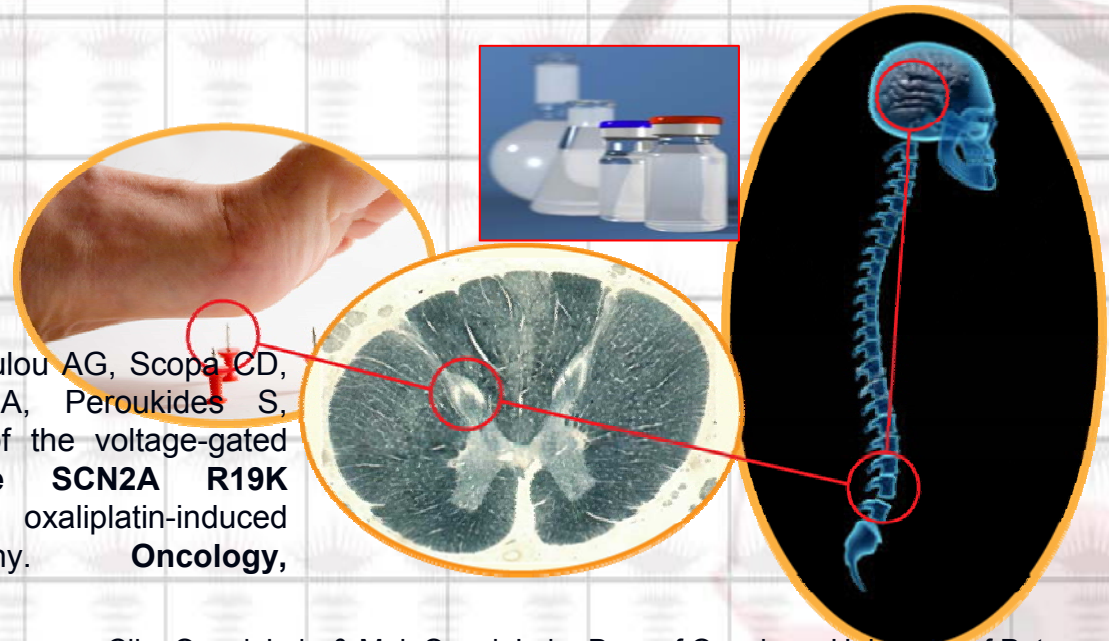
C. Neurotoxicity

Areas of research

In patients treated with highly neurotoxic chemotherapeutic agents is performed:

- Monitoring of peripheral neuropathy
- Study of symptomatic treatment and neuroprotection against peripheral neuropathy
- Investigation on the individual susceptibility at the molecular level
- Identification of predictive surrogate markers

Argyriou AA, Antonacopoulou AG, Scopa CD, Kottorou A, Kominea A, Peroukides S, Kalofonos HP. Liability of the voltage-gated sodium channel **gene SCN2A R19K polymorphism** to oxaliplatin-induced peripheral neuropathy. **Oncology**, 2009;77:254-6.



Future Perspectives

Currently, our studies are focused on:

- ✓ Activation of pathways that contribute to **cancer cell resistance**:
 - Interaction of growth factor receptors with other receptors such as **integrins** that affect cell functions ...
 - Targeting of **intracellular molecules** (PI3K, mTOR, ...) in tumors.
 - Investigation of the **RANK-L/RANK system** in normal breast and **breast cancer** cells.
- ✓ New biomarkers for early cancer diagnosis, prediction and prognosis
- ✓ Molecular markers implicated to neurotoxicity

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